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Endoglin Selectively Modulates Transient Receptor Potential Channel Expression in Left and Right Heart Failure

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Abstract

Introduction—Transient receptor potential (TRP) channels are broadly expressed cation channels that mediate diverse physiological stimuli and include canonical (TRPC), melastatin (TRPM) and vanilloid (TRPV) subtypes. Recent studies have implicated a role for TRPC6 channels as an important component of signaling via the cytokine, transforming growth factor beta 1 (TGFb1) in right (RV) or left ventricular (LV) failure. Endoglin is a transmembrane glycoprotein that promotes TRPC6 expression and TGFb1 activity. No studies have defined biventricular expression of all TRP channel family members in heart failure.

Hypothesis—We hypothesized that heart failure is associated with distinct patterns of TRP channel expression in the LV and RV.

Methods—Paired viable left (LV) and right (RV) ventricular free wall tissue was obtained from human subjects with end-stage heart failure (n=12) referred for cardiac transplantation or biventricular assist device implantation. Paired LV and RV samples from human subjects without heart failure served as controls (n=3). To explore a functional role for endoglin (Eng) as a regulator of TRP expression in response to RV or LV pressure overload, wild-type (Eng+/+) and Eng haploinsufficient (Eng+/−) mice were exposed to thoracic aortic (TAC) or pulmonary arterial (PAC) constriction for 8 weeks. Biventricular tissue was analyzed by real-time polymerase chain reaction.

Results—Compared to non-failing human LV and RV samples, mRNA levels of TRPC1, 3, 4, 6 and TRPV-2 were increased and TRPM2, 3, and 8 were decreased in failing LV and RV samples. TRPC1 and 6 levels were higher in failing RV compared to failing LV samples. After TAC, murine LV levels of TPRC1 and 6 were increased in both Eng +/+ and Eng +/− mice compared to sham controls. LV levels of TRPC4; TRPM3 and 7; TRPV2 and 4 were increased in Eng +/+, not Eng +/− mice after TAC. After PAC, all TRP channel family members were increased in the RV, but not

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LV, of Eng +/+ compared to sham controls. In contrast to Eng+/+, PAC did not increase RV or LV levels of TRP channels in Eng +/− mice.

Conclusions—This is the first study to demonstrate that TRP channels exhibit distinct profiles of expression in the LV and RV of patients with heart failure and in murine models of univentricular pressure overload. We further introduce that the TGFb1 co-receptor endoglin selectively regulates expression of multiple TRP channels in the setting of LV or RV pressure overload.

Keywords

Heart failure; Transient receptor potential channels; Right ventricular failure; Endoglin

1. Introduction

Heart failure is a major cause of morbidity and mortality for nearly 24 million individuals worldwide (Bui et al., 2011). While much attention has focused on signaling mechanisms regulating left ventricular (LV) remodeling, the negative impact of right ventricular (RV) dysfunction on survival remains a significant problem for patients with left heart failure or lung disease (Aronson et al., 2013; Ghio et al., 2001; Gulati et al., 2013; Iglesias-Garriz et al., 2012; McLaughlin et al., 2009). However, several lines of evidence suggest that the RV and LV have distinct profiles of response to injury including: 1) the developmental origin of the RV from a heart field distinct from the LV; 2) a thin RV free wall with susceptibility to increased wall stress; 3) a greater dependence of RV stroke volume on afterload; and 4) enhanced RV contractile resilience to pressure overload (Bogaard et al., 2009; Bristow et al., 1998; Rockman et al., 1994; Urashima et al., 2008; Zaffran et al., 2004). Our understanding of the mechanisms governing RV remodeling stem primarily from data generated in models of LV failure. The identification of molecular targets that improve LV and RV function remains a significant unmet need for patients suffering from heart failure and lung disease.

Transient receptor potential (TRP) channels are broadly expressed cation channels that mediate diverse physiological stimuli and include canonical (TRPC), melastatin (TRPM) and vanilloid (TRPV) subtypes (Clapham, 2003). TRP channels are emerging as key mediators of cardiac hypertrophy and fibrosis, however little is known about TRP channel family expression in HF. Several recent studies implicate TRPC-6 as a central mediator of signaling via transforming growth factor beta 1 (TGFb1). In these studies, TGFb1 promotes expression of calcineurin, which increases levels of TRPC-6, which triggers calcium influx and subsequent calcineurin activation, thereby setting up a self-propagating mechanism for pathologic LV hypertrophy, fibrosis, and increased mortality in heart failure (Eder and Molkentin, 2011; Koitabashi et al., 2010; Kuwahara et al., 2006; Patel et al., 2010). Expression of TRP channel family members in heart failure remains poorly understood.

Endoglin is a 180kDA trans-membrane glycoprotein that promotes TGFb1 signaling in heart failure (Kapur et al., 2010; Kapur et al., 2012). Endoglin null mice die at embryonic day 10.5 due to impaired cardiovascular development and extra-embryonic angiogenesis (Li et al., 1999). In contrast, endoglin heterozygous mice ($Eng^{+/-}$) are viable and have reduced total body levels of endoglin. We recently reported that loss of the TGFb1 co-receptor,

endoglin, attenuates increased TRPC-6 expression, reduces RV fibrosis and improves survival in murine models of RV failure (Kapur et al., 2014). Whether endoglin regulates expression of other TRP channel family members is not known. Based on these background data, the purpose of this study was to determine TRP channel expression in the LV and RV from patients with end-stage HF and to explore whether endoglin regulates expression of TRP channels in response to LV or RV pressure overload.

2. Methods

2.1 Human samples

Paired viable left (LV) and right (RV) ventricular free wall tissue was obtained from human subjects with end-stage HF (n=12) referred for orthotopic heart transplantation (OHT) or biventricular assist device implantation (BIVAD). Non-failing LV and RV tissue obtained from the National Disease Research Interchange (NDRI) served as controls (n=3). All tissue was immediately frozen in liquid nitrogen and stored at −80°C until further processing as described below. All surgical procedures and tissue harvesting were performed in concordance with the National Institutes of Health and Tufts University Institutional Review Board guidelines.

2.2 Surgical models of heart failure

Animals were treated in compliance with the Guide for the Care and Use of Laboratory Animals (National Academy of Science). Animal protocols were approved by the Tufts Medical Center Institutional Animal Care and Use Committee. As described previously, adult, male, 12–14 week old C57BL/6 wild-type (Eng+/+) and Eng+/− mice underwent constriction of the thoracic aorta (TAC) or pulmonary artery (PAC) for 8 weeks to generate models of left HF or right HF, respectively (Kapur et al., 2013; Kapur et al., 2012). Sham-operated mice served as controls (n=6). Ten weeks after TAC or PAC, terminal hemodynamics were recorded using biventricular conductance catheters as previously described ()(Kapur et al., 2013) and both RV and LV tissue obtained for further analysis by real-time polymerase chain reaction (RT-PCR).

2.3 PCR

Total RNA was extracted from the LV and RV with Trizol (Life Technologies) and converted to cDNA with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). PCR was performed in triplicate using 40 cycles at 94° C for 15 seconds, 60° C for 30 seconds and 72° C for 30 seconds with an ABI Prism 7900 Sequence Detection System. The primers used for detection are shown in Table 2.

2.4 Statistics

All statistical analyses were performed using Graph Pad Prism v6 (Graph Pad Software, Inc.). Comparison between two experimental groups was performed with the unpaired student’s T test and for three groups or more with a one-way ANOVA. α values less than 0.05 were accepted as statistically significant.

3. Results

3.1 TRP channel expression in human heart failure

To determine whether TRP channel expression is altered in patients with heart failure, LV and RV samples were obtained from patients with advanced heart failure referred for OHT (n=9) or BIVAD support (n=3; Table 1). Non-ischemic cardiomyopathy was the primary etiology for heart failure in 11 subjects. Compared to non-failing control LV and RV samples, levels of TRPC1, 3, 4 and 6 mRNA were increased in the failing LV and RV respectively (Figure 1). Levels of TRPC1 and 6 mRNA were higher in the failing RV than the failing LV. TRPV1 and 3 were not detectable in the human samples. TRPV2 levels were similarly increased in the failing LV and RV. Levels of TRPM 1,6, and 7 mRNA were not detectable in the LV or RV. Levels of TRPM2, 3 and 8 were reduced to a similar extent in the failing LV and RV compared to non-failing control LV and RV samples. TRPC2 is a pseudo-gene in humans and was not analyzed (Clapham, 2003). TRPC5 and 7 were not detectable in the human LV or RV. Compared to non-failing human LV and RV samples, Endoglin mRNA levels were increased in the LV and RV (Figure 2).

3.2 TRP channel expression in LV pressure overload

To explore whether isolated LV failure altered TRP channel expression and the effect of endoglin haploinsufficiency on TRP channel expression, we employed the well-established model of LV pressure overload induced by trans aortic constriction (TAC) in WT and endoglin haploinsufficient mice (Eng+/−). Compared to sham controls and WT mice after TAC, both RV and LV mRNA levels of endoglin are lower in Eng+/− mice (Figure 2). In sham-operated controls, TRPC-1, 3, 4 and 6; TRPV-2, and 4; and TRPM-3, 4, 6 and 7 are expressed in the LV and RV. Compared to sham-operated controls, LV pressure overload increased LV mRNA levels of TRPC-1, 4, and 6; TRPM-3, 7; and TRPV-2 and 4 levels (Figure 3). TRPM-4 and TRPM-6 levels were unchanged with TAC in the LV and RV (data not shown). LV pressure overload only increased RV mRNA levels of TRPC-4 (Figure 4). Compared to sham-operated controls, LV pressure overload increased LV mRNA levels of TRPC1 and TRPC 6 only in Eng+/− mice (Figure 3). No change in RV mRNA levels of any TRP channels were observed after LV pressure overload in Eng+/− mice. Compared to WT mice subjected to TAC, LV mRNA levels of TRPC-4, TRPM-3, TRPV-2, and TRPV-4 were lower in Eng+/− mice after TAC. We observed increased LV peak systolic pressure in Eng +/− mice, not Eng +/+ mice, following TAC (Figure 3D). LV end diastolic pressure was similarly increased in Eng +/+ and Eng +/− mice after TAC (Figure 3E). LV and RV stroke volume was decreased in Eng +/+ mice and preserved in Eng +/− mice (Figure 3F). These findings indicate LV function was improved in Eng +/− mice compared to WT mice following TAC.

3.3 TRP channel expression in RV pressure overload

To explore whether isolated RV failure altered TRP channel expression, we employed the well-established model of RV pressure overload induced by pulmonary artery constriction (PAC). Compared to sham controls and WT mice after PAC, both RV and LV mRNA levels of endoglin are lower in Eng+/− mice (Figure 2). Compared to sham controls, RV pressure overload increased RV mRNA levels of all TRP channels studied (TRPC-1, 3, 4, 6;

TRPM-3, 7; and TRPV-2, and 4). TRPM-4 and TRPM-6 levels were unchanged with PAC in the LV and RV (data not shown). RV pressure overload did not induce expression of any TRP channel studied in the LV (Figure 4). Next, to explore whether endoglin regulates TRP channel expression in RV pressure overload, TRP mRNA expression was studied in Eng+/− mice subjected to PAC. Compared to sham-operated controls, RV pressure overload did not increase RV or LV mRNA levels of any TRP channel in Eng +/− mice (Figure 4). Compared to WT mice subjected to TAC, RV mRNA levels of all TRP channels were higher after PAC and LV mRNA levels were unchanged. We observed increased RV systolic pressure (RVSP) and no change in end-diastolic pressure in both Eng +/+ and Eng +/− mice (Figure 4D–E). Despite equally increased RVSP in both Eng +/+ and +/− mice, LV and RV stroke volume was decreased in Eng +/+, not Eng +/−, mice (Figure 3F). Compared to WT mice, these findings suggest RV function was improved in Eng +/− mice following PAC.

4. Discussion

Our central finding is that biventricular TRP channel expression is altered in heart failure. Specifically, we report the following: 1) mRNA levels of multiple TRP channel family members are increased in the LV and RV of patients with advanced heart failure, 2) LV pressure overload increases LV and RV mRNA levels of multiple TRP channels in a murine model of thoracic aortic constriction, 3) RV pressure overload increases RV, not LV, mRNA levels of multiple TRP channels, and 4) reduced endoglin expression attenuates increased mRNA levels of multiple TRP channels after LV or RV pressure overload. Our findings suggest that further exploration of the role of TRP channels in biventricular remodeling may identify novel targets of therapy for heart failure or pulmonary hypertension and further introduces that the TGFb1 co-receptor, endoglin, regulates biventricular TRP channel expression.

The TRPC family has been well described as mediators of pathological cardiac remodeling. TRPC-1,3,4 and 6 are mechanosensitive mediators of calcium influx into cardiomyocytes which activate NFAT/calcineurin signaling and downstream maladaptive signaling as well as promote transformation of fibroblasts into highly secretory myofibroblasts (Seth 2009, Camacho Londono 2015, Seo PNAS 2014). TRPC loss of function studies using genetic and pharmacological approaches have shown suppression of pressure overload induced heart failure in mouse models (Seth 2009, Camacho Londono 2015, Seo PNAS 2014). We observed increased levels of TRPC (1, 3, 4, 6) in failing human samples and mouse models of pressure overload induced heart failure. These data further substantiate that TRPC channels are upregulated in human heart failure and suggest targeting TRPC channel expression or activity may ameliorate heart failure.

The role of TRPV and TRPM channels in human HF is not well defined.. Prior studies have established expression of specific TRPM (3,7) and TRPV (2,4) subtypes in human cardiac fibroblasts and suggest calcium influx mediated by these channels contribute to myofibroblast transformation (Adapala et al., 2013; Du et al., 2010; Iwata et al., 2013). We found increased levels of TRPV2 and reduced levels of TRPM 2,3, and 8 in the failing LV and RV. Functional TRP channels assemble from homomeric and heteromeric oligomerization of TRPC, TRPM and TRPV subunits (Alessandri-Haber et al., 2009;

Hofmann et al., 2002; Kobori et al., 2009; Poteser et al., 2006). The subunit composition of TRP channels in HF is not well established and TRPV or TRPM may participate in the formation of functional TRP channels underlying pathological cardiac remodeling. Future studies may focus on the functional significance of TRPV and TRPM transcriptional regulation in HF.

RV failure is an unmet clinical need without specific medical therapy. All of the examined TRPC channels were upregulated in the human failing LV and RV. We observed profound upregulation of the examined TRPC channels in the pressure-overloaded RV and severely impaired RV systolic function. Our findings indicate TRPC channel upregulation may be a molecular signature of the failing RV and a potential therapeutic target. Development of pharmacological inhibitors targeting TRP subtypes in prior pre-clinical studies of pressure overload and post-myocardial infarction HF has shown limited progress to date, in part due to the inherent difficulty of modulating multiple TRP channels with a single agent (Camacho Londono et al., 2015; Makarewich et al., 2014; Seo et al., 2014). Endoglin deficiency attenuated TRP channel upregulation among all subtypes in the pressure overloaded RV and improved RV function.We have previously shown reduction of endoglin activity limits TRPC6 mediated calcineurin activation and fibrosis in the failing RV (Kapur et al., 2014). Further studies are necessary to establish if the salutatory functional effects of endoglin blockade in the pressure overloaded RV are attributable to modulation of TRP channel expression and/or non-TRP mediated signaling pathways.

Prior studies of TRP channel expression in the failing heart have reported disparate findings. TRPV2 and TRPC6 have been alternately reported as not detectable, unchanged or increased in the LV of patients with dilated cardiomyopathy (Iwata et al., 2013; Kuwahara et al., 2006; Watanabe et al., 2009). In part the lack of agreement may be explained by methodological differences including sample location (atria vs. ventricle), patient characteristics (non-ischemic vs. ischemic cardiomyopathy), and sample type (isolated cell preparations vs. tissue homogenates). We sought to examine differential mRNA expression of the TRPC, TRPV and TRPM families in the failing LV and RV.

Our study is limited by the availability of commercially available antibodies with sufficient specificity to differentiate between TRP channel subtypes in mice and humans and therefore we employed real time PCR as performed in contemporaneous studies (Makarewich et al., 2014; Seo et al., 2014). Without measurement of TRP channel protein levels, we cannot determine if the observed differences are manifest at the post-transcriptional level. As reliable and specific antibodies for the TRP channel subtypes are developed, future studies may permit detailed cell and TRP subunit specific analysis of the functional role of TRP channels in adverse cardiac remodeling.

5. Conclusions

In conclusion, heart failure is a major cause of global mortality. Limited studies have explored the expression profile of TRP channels in heart failure. Recent reports have suggested a link between TGFb1, endoglin, and TRPC-6 as mediators of cardiac fibrosis in right and left heart failure. We now introduce that biventricular expression of multiple TRP

channels is altered in patients with heart failure and that LV or RV pressure overload generate distinct profiles of TRP expression. Furthermore, we show that intact reduced endoglin expression prevents upregulation of TRP channels in murine models of right or left ventricular pressure overload. Our studies support that TRP channels may represent novel targets of therapy for heart failure.

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Highlights

- A distinct TRP expression profile was observed in advanced human heart failure
- Select TRP channel expression is higher in the failing RV than LV
- TRP channel upregulation requires full endoglin activity in the failing RV

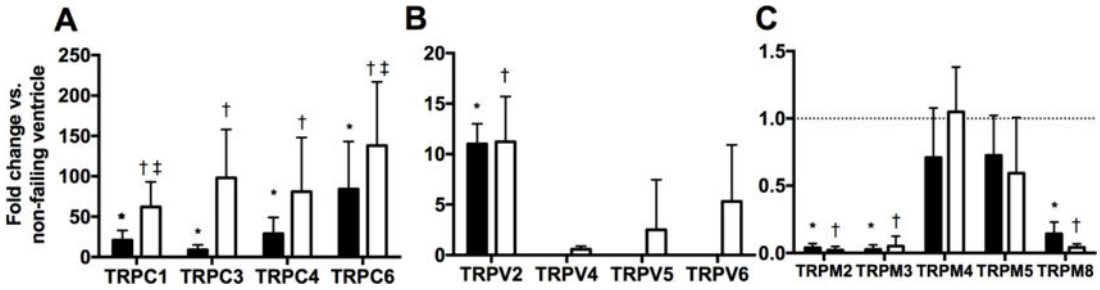

Figure 1. Distinct expression of RV and LV TRP channels in human subjects with advanced heart failure

mRNA expression levels of TRPC (A), TRPV (B) and TRPM (C) in human LV (closed bars) and RV (open bars) samples. Data are expressed as fold change compared to normal ventricle. $p<0.05$: *, vs Normal LV; †, vs Normal RV; ‡, vs Failing LV. ND=not detected.

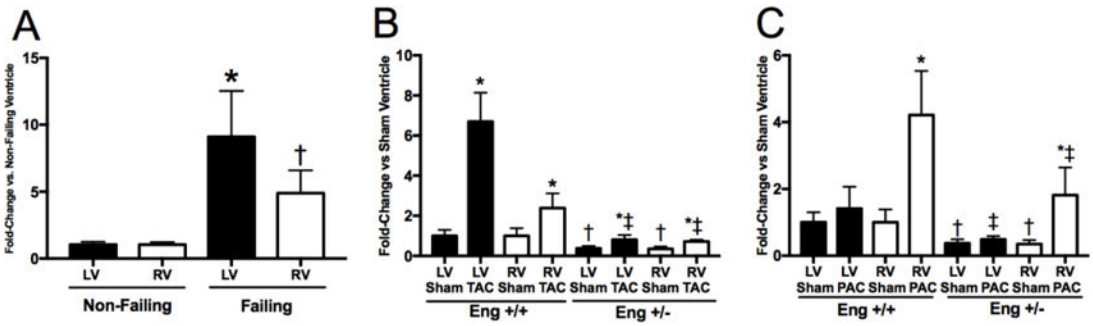

Figure 2. Endoglin mRNA expression following 8 weeks of LV or RV pressure overload

mRNA expression levels of Endoglin in human LV and RV (A). Data are expressed as fold change compared to non-failing ventricle. $p<0.05$: *, vs Non-Failing LV; †, vs Non-Failing RV. mRNA expression levels of Endoglin in the LV (closed bars) and RV (open bars) following 8 weeks of TAC (B) or PAC (C).*, vs. Sham operated corresponding ventricle; †, vs. Eng +/+ Sham operated corresponding ventricle; ‡, vs. Eng +/+ TAC or PAC corresponding ventricle

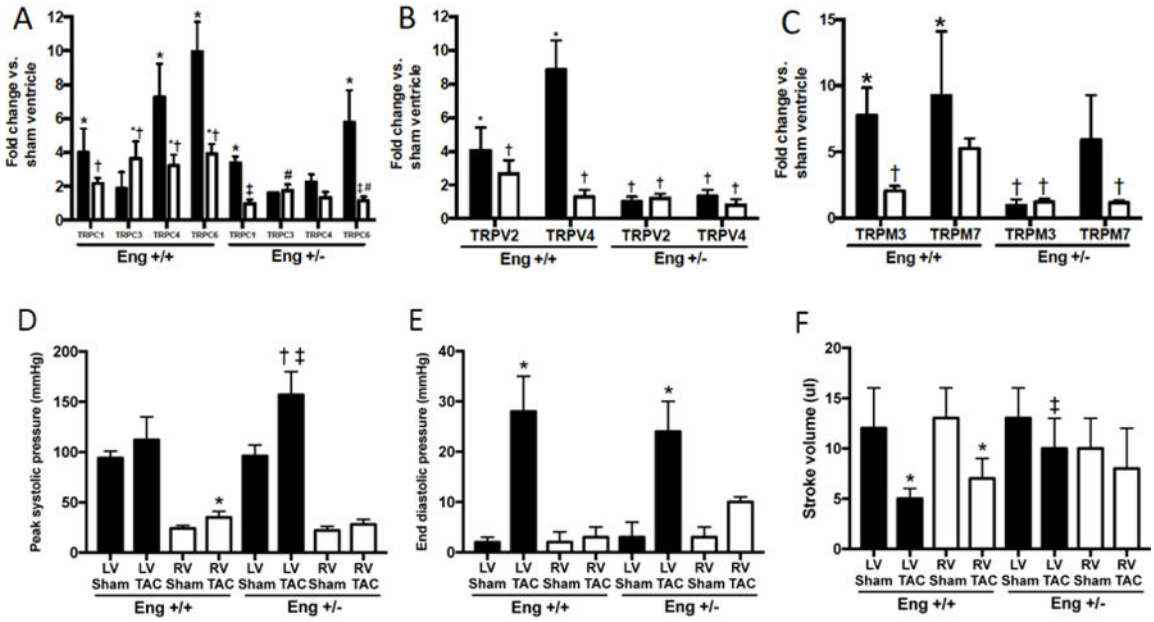

Figure 3. Biventricular TRPC expression and hemodynamics following 8 weeks of LV pressure overload

mRNA expression levels of TRPC (A), TRPV (B) and TRPM (C) in the LV (closed bars) and RV (open bars) following 8 weeks of TAC. Data are expressed as fold change compared to sham operated ventricle. $p<0.05$: *, vs. Sham operated corresponding ventricle; †, vs. Eng +/+ TAC LV; ‡, vs. Eng +/− TAC LV; #, vs. Eng +/+ TAC RV; %, vs. Eng +/− TAC RV.Peak systolic pressure (D), end diastolic pressure (E) and stroke volume (F) were measured under steady state conditions. $p<0.05$: *, vs. Sham operated corresponding ventricle; †, vs. Eng +/+ Sham operated corresponding ventricle; ‡, vs. Eng +/+ TAC corresponding ventricle

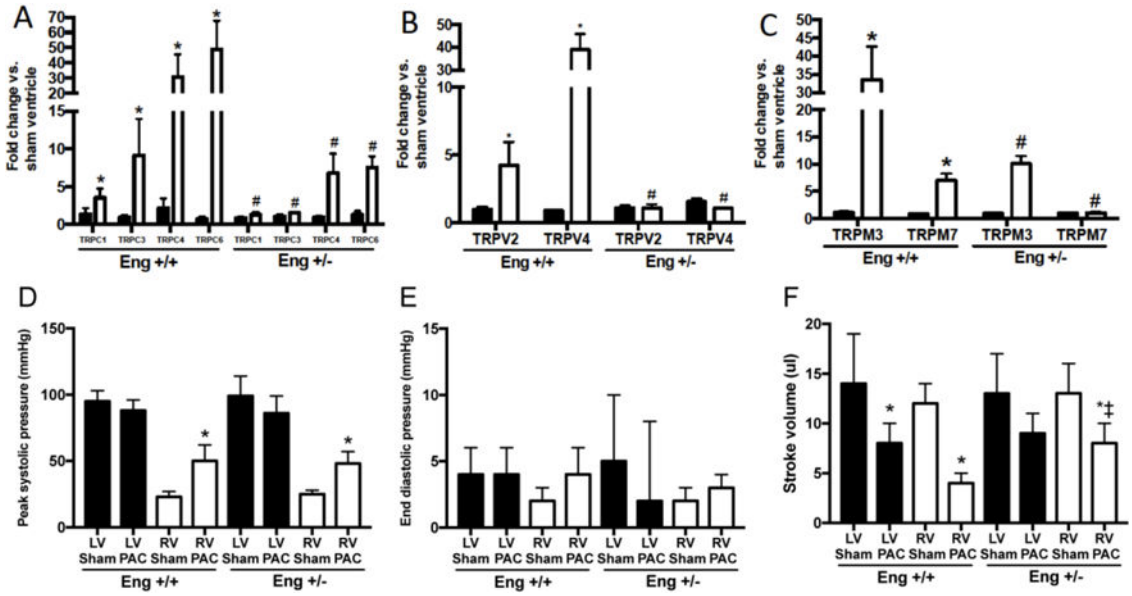

Figure 4. Biventricular TRP expression and hemodynamics following 8 weeks of RV pressure overload

mRNA expression levels of TRPC (A), TRPV (B) and TRPM (C) in the LV (closed bars) and RV (open bars) following 8 weeks of PAC. Data are expressed as fold change compared to sham operated ventricle. $p<0.05$: *, vs. Sham operated corresponding ventricle; †, vs. Eng +/+ PAC LV; ‡, vs. Eng +/− PAC LV; #, vs. Eng +/+ PAC RV; %, vs. Eng +/− PAC RV. Peak systolic pressure (D), end diastolic pressure (E) and stroke volume (F) were measured under steady state conditions. $p<0.05$: *, vs. Sham operated corresponding ventricle; †, vs. Eng +/+ Sham operated corresponding ventricle; ‡, vs. Eng +/+ TAC corresponding ventricle

Table 1

Clinical characteristics of patients with advanced heart failure

Patient	Age	Gender	Cardiomyopathy	Indication for surgery
1	20	F	NICM	OHT for post-partum cardiomyopathy
2	52	F	NICM	OHT for NICM post-LVAD
3	64	M	NICM	OHT for HCM
4	53	F	NICM	BIVAD for myocarditis
5	45	M	NICM	BIVAD for myocarditis
6	34	M	NICM	OHT for NICM post-LVAD
7	50	F	NICM	OHT for NICM
8	53	F	NICM	OHT for chemotherapy related NICM post-LVAD
9	44	F	NICM	OHT for giant cell myocarditis
10	64	M	ICM	OHT post-LVAD
11	56	M	NICM	BIVAD for myocarditis
12	67	F	NICM	OHT for chemotherapy related NICM

Table 2

Primers used for real time PCR analysis References

Mouse	Forward primer	Reverse primer
Endoglin	ctgccaatgctgtgcgtgaa	gctggagtcgtaggccaagt
TRPC1	ctgcgaacagcaaagcaatg	gaagatgtaccagaacagagc
TRPC3	ggtggtcgttttactcaacatgc	catcgaagtaggagagccaaag
TRPC4	gactatgacttaagccccacgg	gatttcaaacattttgcctgcc
TRPC6	cacagaagacctagcagagctca	ataatatggcttcaagtggagaaat
TRPV2	cggaccagcaagtacctcactg	ccggaatccctgtcaatctg
TRPV4	ccctggcaagagtgaaatctacc	catctgcgcttgagttcttgttc
TRPM3	atcgcttcaattcgtccaacg	tgctagccggatgtccacg
TRPM4	ccaacactgttctcgagtcctgac	gcaaacacctagacatccaccag
TRPM6	ctgacctcttggccttcacttac	ggaaaaggtgcttagactgtctaag
TRPM7	gaagagcagtgtgttgagatgtac	tatgtagttgacacgatctccaac
Human	Forward primer	Reverse primer
TRPC1	tatggatgttgcacctgtcattt	actgggagacaaactctttctgg
TRPC3	acgacttctacgcttacgacgag	cttaatggcaagtttgacacgac
TRPC4	gaggtactctgcctactcccttca	gagccattgcttatgttatgtcttt
TRPC5	caatgtgaaagccagacacgaat	tctatttcccaagaggtcaagca
TRPC6	ttgacgaaagtaacattgggagac	accagattgaagggtacaggaag
TRPC7	ttgtggaacctgctagatttcgg	ggttgtatttggcacctcggtag
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